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Phytochemical and Antibacterial Investigation on Dendrophthoe Falcata (L.F.) Ettingsh Growing on Toona Serrata (Royle.) Roem.

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ABSTRACT

The present investigation is designed to evaluate phytochemical contents, qualitatively and quantitatively and to study antibacterial activity of Dendrophthoe falcata (L.f.) Ettingsh belonging to family Loranthaceae growing on the host plant Toona serrata (Royle.) Roem. of family Meliaceae. The preliminary phytochemical tests were performed in five different solvents viz. Ethyl acetate, Chloroform, Distilled water, Methanol and Ethanol, which showed the presence of Carbohydrates, proteins, Glycosides, steroids, saponins, alkaloids, flavonoids, phenolics, etc. The ethanolic extract of Dendrophthoe falcata (L.f.) Ettingsh showed strong antibacterial activity against the micro-organisms i.e. S. aureus, E. coli and P. aeruginosa, while the methanolic extract and aqueous extract showed moderate activity.

Keywords: Dendrophthoe falcata (L.f.) Ettingsh, Toona serrata (Royle.) Roem., antibacterial activity, S. aureus, E. coli, P. aeruginosa, alkaloids, flavonoids, phenolics, etc.

I. INTRODUCTION

The genus Dendrophthoe is evergreen, shrubby partial parasitic plant distributed in the tropical and subtropical regions of the world. The whole parasitic plant is used in indigenous system of medicine as cooling, bitter tonic, astringent, aphrodisiac, narcotic, diuretic, pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesicle calculi and vilated conditions of kapha and pitta (Nadkarni, 1993). Dendrophthoe falcata belonging to the family Loranthaceae is an angiospermic hemiparasitic plant was most frequently observed on many host plants, comprises of 20 species and about 7 species are found in India. Dendrophthoe falcata is reported to contain biological active substances such as flavonoid, quercetin, kempferol, rutin (Ramchandran et al. 1999), tannins, β-sitosterol, stigmasterol, β-amyrin, oleanolic acid (Anjaneyula et al. 1993).

II. MATERIALS AND METHODS

Plant material

The Dendrophthoe falcata leaves and stem was collected from the host Toona serrata (Royle.) Roem. during March- April of 2015 from Melghat forest region of West Vidarbha, Maharashtra, India and were authenticated by Dr. S. P. Rothe, Head, Department of Botany. The herbarium specimens were given voucher number and deposited in the Department of Botany, Shri Shivaji College of Arts, Commerce and Science, Akola, Maharashtra, India.

Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary and secondary metabolites (Harborne, 1973).

Tests for carbohydrates

Fehling's Test: 1ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitate was observed.

Test for Proteins

Biurret Test: To the small quantity of extract 1-2 drops of Biurret reagent was added. Formation of violet colour precipitate showed presence of proteins.

Test for Anthraquinone glycosides

Borntrager's Test: To the 3ml of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

Test for Cardiac glycosides

Keller-Killiani Test: To the 5ml extract, 1 ml of conc. H_2SO_4, 2 ml of Glacial acetic acid and 1 drop of $FeCl_3$ solution was added. Appearance of brown ring shows the presence of cardiac glycosides.

Test for Coumarins

To the 2ml extract, 10% NaOH was added and shaken well for 5 min. which shows yellow colour.

Test for Quinone

To the 2ml extract conc. H_2SO_4 was added and shaken well for 5 min. The appearance of Red colour confirmed the presence of quinones.

Test for Steroids

Salkowski Test: To 2 ml extract, 2ml of Chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result, chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for Alkaloids

Dragendroff's Test: To the 2-3ml of filtrate, few drops of dilute HCl and few drops of Dragendroff's reagent was added and shaken well. Formation of orange-brown precipitate confirms the presence of alkaloids.

Test for Flavonoids

Shinoda test: To the 2ml filtrate, 5 ml of 95% ethanol and few drops of conc. HCl was added. To this solution 0.5gm of magnesium turnings were added. Appearance of pink colouration indicates the presence of flavonoids.

Test for Phenolics and Tannin

$FeCl_3$ solution test: On addition of 5% $FeCl_3$ solution to the plant extract, deep blue-black colour appeared showing the presence of Phenolics.

Test for Saponin

Foam Test: To 1ml extract 20ml distilled water was added and shaken well in test tube for 10 min. Foam or lather formation showed the presence of saponins

Test for Terpenoids

2ml of extract was thoroughly shaken with 2ml chloroform and then 3ml conc. H_2SO_4 was added through walls of test tube. Reddish brown colour appeared which indicated the presence of terpenoids.

Quantitative Phytochemical Analysis

The crude quantification of major phytochemicals present was done using precipitation and spectrophotometric method as per suitability. Each sample was analysed in triplicates. Only Alkaloids, Flavonoids, Saponins and Phenolics were quantified by the standard procedures given below.

Alkaloids

5 gm of sample was weighed in 250 ml beaker and 200 ml 20% acetic acid in ethanol was added and covered to stand for about 4 hrs. This was filtered and extract was concentrated using water bath to $1/4^{th}$ of original volume. Concentrated Ammonium hydroxide was added drop wise to the extract till its complete precipitation. The whole solution was allowed to settle and precipitate was collected and weighed (Harborne, 1973).

Flavonoids: 10 gm of sample was extracted repeatedly in 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman paper no. 42. The filtrate then transferred to a crucible and evaporated to dryness over a water bath and weighed (Bohm and Kocipai- Abyazan, 1994).

Phenolics

The total phenolics in the extract were determined using Folin-Ciocalteu method. To each sample solution (1.0 ml) and standard (Gallic acid) was added 5 ml of Folin-Ciocalteu and 4 ml sodium carbonate (7 % w/v). The mixture was shaken and allowed to stand for 30 min in the dark at room temperature; after which absorbance was measured at 765 nm using a spectrophotometer. The amount of total phenolics was expressed as Gallic acid equivalent (GAE) in milligram per gram dry plant extract using the expression; C = c x (V/m); (where C= Total phenolics content of plant extract in mg/g GAE, c= concentration of Gallic acid established from calibration curve mg/g, V= volume of the extract (ml) and m= weight of pure plant extract (g) (Vermerris et al., 2006).

Saponin

10 gm of plant powder was taken in 200 ml 20% ethanol to make a suspension. This was heated for about 4 hrs over hot water bath (55^oC) continuous stirring. The mixture was filtered and the residue was re-extracted with 200 ml 20% ethanol. The combined extract was reduced to $1/10^{th}$ of the original volume. The concentrate was taken into 250 ml separating funnel, to this added 20 ml diethyl ether and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated for 2-3 times. Then 60 ml n-butanol was added to it. The combined solution was then washed twice with 10 ml 5% aqueous sodium hydroxide. The remnant was heated in a water bath for complete evaporation and dried. This dried content was calculated as Saponin amount in a sample (Obdoni & Ochuko, 2001).

Extraction procedure

Shade dried leaves were finely powdered and subjected to successive solvent extraction by continuous Soxhlet extraction. The extraction was done with different solvents with respect to their increasing order of polarity such as Ethyl acetate, Chloroform, Methanol, Ethanol and water. All the extracts were concentrated by distilling the solvent in a rotary evaporator. The dried extracts were dissolved in respective solvents with concentration 2mg/ml and subjected to antibacterial activity.

Test organisms

The bacterial spp. used for the test were Staphylococcus aureus (S. aureus), Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa). All the stock cultures were obtained from Microbiology lab, Shri Shivaji College of Arts, Commerce and Science, Akola, Maharashtra, India.

Culture media and inoculums preparation

Nutrient agar /broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth at 32^0C for 72 hrs.

Antibacterial activity

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic penicillin (100 μg/mL) in-vitro by disc diffusion method (Laouer et al., 2009) using S. aureus, E. coli and P. aeruginosa as test organisms. Each extract was individually loaded on the 3mm sterile disc at the concentration of 2 mg/ml. The results were recorded by measuring the zone of growth inhibition surrounding the disc. The experiments were done in triplicates.

III. RESULTS AND DISCUSSION

The leaf and stem powder were tested for the phytochemicals present in five solvents viz. Ethyl acetate, Chloroform, Methanol, Ethanol and Distilled Water. Methanolic and ethanolic extracts showed presence of more phytochemicals than rest of the solvents. Also, the presence of phytochemicals was found to be more and abundant in leaf than in the stem. The screening results of the preliminary phytochemical tests are enumerated in Table 1.

The leaf and stem powders were also subjected for quantitative estimation of Alkaloids, Flavonoids, Phenolics and Saponin. Leaf showed greater quantity of phytochemicals as compared to that in stem. The values of quantitative estimation of Dendrophthoe falcata (L.f.) Ettingsh are showed in Table 2.

The leaf and stem extracts of Dendrophthoe falcata (L.f.) Ettingsh were tested for antibacterial activity against three pathogenic bacteria i.e. E. coli, P. aeruginosa and S. aureus and compared with the results of the standard antibiotic, zone of inhibition of Penicillin. Methanolic and ethanolic leaf extracts showed more effective zone

of inhibition against all the three pathogenic bacteria, whereas, other leaf extracts showed moderate zone of inhibition. All the stem extracts were seen to be ineffective against E. coli. Ethanolic stem extract showed prominent zone of inhibition against P. aeruginosa and S. aureus while aqueous extract showed moderate activity against the two pathogens. The results of antibacterial activity are put forth in Table 3.

The therapeutic value of medicinal plants lies in the various chemical constituents present in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For example, plants those are rich in phenolics content show remarkable antibacterial potential due to the basic character that allows them to react with proteins, to form stable water-soluble compounds thereby killing the bacteria directly by damaging its cell membrane (Mohamed et al., 2010). Flavonoids are a major group of phenolic compounds reported for their antiviral (Mehrangiz et al., 2011), antimicrobial (Maria and Maria., 2009) and spasmolytic properties (Julianeli et al., 2011). Alkaloids isolated from plants are in general are found to have antimicrobial properties (Ahmed et al., 2010). The antibacterial activity of D. falcata leaves, therefore, can be attributed to the presence of phytochemicals viz. flavonoids, alkaloids, phenolics, glycosides in methanolic, ethanolic and aqueous extracts.

IV. CONCLUSION

It is concluded that the Dendrophthoe falcata (L.f.) Ettingsh plant extract possesses antibacterial activity against tested organisms. The zone of inhibition varied, suggesting the varying degree of efficacy and different phytoconstituents of herb on the target organism. The antibacterial activity of the plants may be due to the presence of various active principles in the leaf and stem of Dendrophthoe falcata (L.f.) Ettingsh. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs.

V. REFERENCES

[1]. Ahmed el-H.M., Nour B.Y., Mohammed Y.G., Khalid HS. (2010). Antiplasmodial activity of some medicinal plants used in Sudanese folk-medicine. Env Health Insts.; 4(4): 1-6.

[2]. Anjaneyula, L.Row and R. Ramhandra. (1993). Chemical constituents of Loranthus falcatus Linn.f. Curr. Sci.1993;46(24):850-851.

[3]. Bohm B.A., Kocipai- Abyazan R. (1994). Flavonoid and condensed tannins from the leaves of Vaccinum raticulation and Vaccinum calcyimium. Pacific Sci., 48: 458-463.

[4]. Harbone, J.B. (1973) Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis, Chapman and Hall London.

[5]. Julianeli T.D.L., Jackson R.G.S., Kelly S., Ana Silvia S.C. (2011). Selective spasmolytic effect of a new furanoflavoquinone derivative from diplotropin on guinea-pig trachea. J Chem Pharm Res.; 3(1): 249-258.

[6]. Laouer H, Meriem EK, Parado S, Baldovini N. (2009). An antibacterial and antifungal phenylpropanoid from C. montaum. Phytother Res.;23: 1726–1730.

[7]. Maria Lysete A.B., Maria Raquel F.L. (2009). Studies on the antimicrobial activity and brine shrimp toxicity of Z. tuberculosa extracts and their main constituents. Annals of Clil Microb Antimic.; 8: 16.

[8]. Mehrangiz KK, Seyed AE, Masoud SG, Esmaeel AS, Amirhossein S. (2011). Antiviral activities of aerial subsets of Artemisi species against Herpes Simplex virus type 1 (HSV1) in vitro. Asian Biomed.; 5(1): 63-68.

[9]. Mohamed Sham Shihabudeen H, Hansi Priscilla D, Kavitha T. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Int J of Pharma Sci Res.; 1(10): 430-434.

[10]. Nadkarni KM. (1993). Indian Materia Medica. Vol. I. Popular Prakashan Pvt. Ltd. Bombay, 750, 1276- 1277.

[11]. Obadoni B.O., Ochuko P.O. (2001). Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. Global J. Pure Appl. Sci., 8: 203- 208.

[12]. Ramchandran AG and P. Krishanakumary. (1999). Flavonoids of Dendrophthoe falcata Ettingsh growing on different host plants. Ind. J. Chem.; 29:584-585.

[13]. Vermerris W., Nicholson R. (2006). Phenolic compound biochemistry. The Netherlands: Springer, Dordrecht.

Table 1. Preliminary Phytochemical analysis of Dendrophthoe falcata (L.f.) Ettingsh leaf and stem growing on T. serrata extracted with different solvents.

Phytoconstituents	EtE		CE		ME		EE		AE	
	L	S	L	S	L	S	L	S	L	S
Carbohydrates	-	-	-	-	+	+	+	+	+	-
Proteins	-	-	-	-	-	-	-	-	-	-
Anthraquinone Glycosides	-	-	+	-	+	-	+	-	-	-
Cardiac Glycosides	+	-	+	-	+	-	+	-	-	-
Caumarins	-	-	+	-	-	-	+	-	-	-
Quinone	-	-	-	-	-	-	-	-	-	-
Steroids	-	+	+	-	-	-	+	-	-	-
Alkaloids	+	+	+	-	+	+	+	+	+	+
Flavonoids	+	-	+	-	+	-	+	+	+	-
Phenolics & Tannins	+	-	+	-	+	+	+	-	+	+
Saponin	-	-	-	-	+	-	+	-	+	-
Terpenoids	-	+	+	-	-	-	-	-	-	-

EtE: Ethyl acetate extract, CE: Chloroform extract, ME: Methanol extract, EE: Ethanol extract, AE: Aqueous extract, L: Leaf, S: Stem

Table 2. Quantitative Phytochemical analysis of Dendrophthoe falcata (L.f.) Ettingsh leaf and stem growing on T. serrata

Sr. No.	Phytochemicals	Leaf (mg/100g)	Stem (mg/100g)
1	Alkaloid	6.71 (± 0.16)	3.66 (± 0.08)
2	Flavonoid	9.66 (± 0.52)	4.92 (± 0.15)
3	Phenolics	10.21 (± 0.25)	1.21 (± 0.03)
4	Saponin	15.62 (± 0.18)	16.71 (± 0.26)

Table 3. Antibacterial activity of different extracts of leaf and stem of Dendrophthoe falcata (L.f.) Ettingsh growing on T. serrata (Mean ± SEM) (mm).

Antibacterial activity tested for	Zone of Inhibition					
	E. coli		P. aeruginosa		S. aureus	
D. falcata Leaf	EtE	14 ± 0.11	EtE	Nil	EtE	10 ± 0.04
	CE	11 ± 0.07	CE	Nil	CE	15 ± 0.11
	ME	25 ± 0.14	ME	17 ± 0.12	ME	40 ± 0.12
	EE	31 ± 0.15	EE	24 ± 0.13	EE	31 ± 0.11
	AE	Nil	AE	Nil	AE	25 ± 0.10
D. falcata Stem	EtE	Nil	EtE	Nil	EtE	15 ± 0.08
	CE	Nil	CE	Nil	CE	Nil
	ME	Nil	ME	16 ± 0.08	ME	15 ± 0.07
	EE	Nil	EE	26 ± 0.14	EE	37 ± 0.15
	AE	Nil	AE	18 ± 0.11	AE	18 ± 0.11
Penicillin	16.01 ± 0.14		16.09 ± 0.19		16.02 ± 0.18	

EtE: Ethyl acetate extract, CE: Chloroform extract, ME: Methanol extract, EE: Ethanol extract, AE: Aqueous extract, L: Leaf, S: Stem